OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW GUIDELINE:

The Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying Ocular Corrosives and Severe Irritants

INTRODUCTION

1. The Bovine Corneal Opacity and Permeability (BCOP) test method is an *in vitro* test method that can be used, under certain circumstances and with specific limitations, to classify substances as ocular corrosives and severe irritants" as defined by the U.S. Environmental Protection Agency (EPA) (Category 1), the European Union (EU) (Category R41), and the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (Category 1). For the purpose of this Test Guideline, severe irritants are defined as those that induce ocular lesions that persist in the rabbit for at least 21 days after administration. While it is not considered valid as a complete replacement for the *in vivo* rabbit eye test, the BCOP is recommended for use as part of a tiered-testing strategy for regulatory classification and labeling within a specific applicability domain (1)(2). Substances (including formulations) can be classified as ocular corrosives or severe irritants without further testing in rabbits. A substance that tests negative would need to be tested in rabbits using a sequential testing strategy, as outlined in OECD Test Guideline 405 (3).

2. The purpose of this Test Guideline is to describe the procedures used to evaluate the potential ocular corrosivity or severe irritancy of a test substance as measured by its ability to induce opacity and increased permeability in an isolated bovine cornea. Toxic effects to the cornea are measured by: (i), decreased light transmission (opacity); and (ii), increased passage of sodium fluorescein dye (permeability). The opacity and permeability assessments of the cornea following exposure to a test substance are combined to derive an *In Vitro* Irritancy Score (IVIS), which is used to classify the irritancy level of the test substance.

3. Ocular irritants that induce lesions that resolve in less than 21 days and non-irritants have also been tested using the BCOP test method. However, the accuracy and reliability of the BCOP test method for substances in these categories, as defined by the EPA (4), EU (5), and GHS (6), have not been formally evaluated.

4. Definitions are provided in Annex I.

INITIAL CONSIDERATIONS AND LIMITATIONS

5. This Test Guideline is based on the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) BCOP test method protocol, which was developed following an international validation study (1)(2)(7), with contributions from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM). The protocol is based on information obtained from the Institute for In Vitro Sciences (IIVS) and INVITTOX Protocol 124 (8), which represents the protocol used for the European Community-sponsored prevalidation study of the BCOP assay

conducted in 1997-1998. Both of these protocols are based on the BCOP assay methodology first reported by Gautheron et al. (8)(9).

6. The identified limitations for this test method are based on the high false positive rates for alcohols and ketones and the high false negative rate for solids observed in the validation database (see paragraph 44) (2). When substances within these chemical and physical classes are excluded from the database, the accuracy of BCOP across the EU, EPA, and GHS classification systems is substantially improved (2). Based on the purpose of this assay (*i.e.,* to identify ocular corrosives/severe irritants only), false negative rates are not critical since such substances would be subsequently tested in rabbits using a sequential testing strategy. Furthermore, the current validation database did not allow for an adequate evaluation of some chemical or product classes (*e.g.,* formulations). However, investigators could consider using this test method for testing all types of substances (including formulations), whereby a positive result could be accepted as indicative of an ocular corrosive or severely irritating response.

7. All procedures with bovine eyes and bovine corneas should follow the testing facility's applicable regulations and procedures for handling animal-derived materials, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended (10).

8. A limitation of the test method is that, although it takes into account some of the ocular effects evaluated in the rabbit ocular irritancy test method and to some degree their severity, it does not consider conjunctival and iridal injuries. Also, although the reversibility of corneal lesions cannot be evaluated *per se* in the BCOP assay, it has been proposed, based on rabbit eye studies, that an assessment of the initial depth of corneal injury can be used to predict irreversible or reversible corneal effects (11). Finally, the BCOP does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.

9. Efforts are ongoing to further characterize the usefulness and limitations of the BCOP assay for identifying non-severe irritants and non-irritants (see also paragraph 45). This Test Guideline will be updated periodically as new information and data are considered. For example, histopathology may be potentially useful when a more complete characterization of corneal damage is needed. To evaluate this possibility, users are encouraged to preserve corneas and prepare histopathology specimens that can be used to develop a database and decision criteria that may further improve the accuracy of this test method. Users are also encouraged to provide specimens and/or data to validation organizations for a formal evaluation of possible future uses of the BCOP test method, including for the identification of non-severe irritants and non-irritants. The OECD is developing a Guidance Document on the use of *in vitro* ocular toxicity test methods, which will include detailed procedures on the collection of histopathology specimens and information on where to submit specimens and/or histopathology data.

10. For any laboratory initially establishing this assay, the proficiency standards provided in Annex II (*To be provided*) should be followed. A laboratory can use these standards to demonstrate their technical competence in performing the BCOP test method prior to submitting BCOP assay data for regulatory hazard classification purposes.

PRINCIPLE OF THE TEST

11. The BCOP test method is an organotypic model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea *in vitro*. In this test method, damage by the test substance is assessed by quantitative measurements of changes in corneal

opacity and permeability with an opacitometer and an ultraviolet/visible (UV/VIS) spectrophotometer, respectively. Both measurements are used to calculate an IVIS, which is used to assign an *in vitro* irritancy hazard classification category for prediction of the *in vivo* ocular irritation potential of a test substance (see Decision criteria).

12. The BCOP test method uses isolated corneas from the eyes of freshly slaughtered cattle. Corneal opacity is measured quantitatively as the amount of light transmission through the cornea. Permeability is measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea, as detected in the medium in the posterior chamber. Test substances are applied to the epithelial surface of the cornea by addition to the anterior chamber of the corneal holder. Annex III provides a description and a diagram of a corneal holder used in the BCOP. Corneal holders can be obtained commercially from different sources or be constructed.

Source and Age of Bovine Eyes and Selection of Animal Species

13. Cattle sent to slaughterhouses are typically killed either for human consumption or for other commercial uses. Only healthy animals considered suitable for entry into the human food chain are used as a source of corneas for use in the BCOP. Because cattle have a wide range of weights, depending on breed, age, and sex, there is no recommended weight for the animal at the time of slaughter.

14. Variations in corneal dimensions can result when using eyes from animals of different ages. Corneas with a horizontal diameter >30.5 mm and central corneal thickness (CCT) values ≥1100 µm are generally obtained from cattle older than eight years, while those with a horizontal diameter <28.5 mm and CCT <900 µm are generally obtained from cattle less than five years old (13). For this reason, eyes from cattle greater than 60 months old are not typically used. Eyes from cattle less than 12 months of age have not traditionally been used since the eyes are still developing and the corneal thickness and corneal diameter are considerably smaller than that reported for eyes from adult cattle. However, the use of corneas from young animals (*i.e.*, <12 months old) has advantages such as increased availability, a narrow age range, and decreased hazards related to potential worker exposure to Bovine Spongiform Encephalopathy (15). As further evaluation of the effect of corneal size or thickness on responsiveness to corrosive and irritating substances would be useful, users are encouraged to report as much information as possible on the age, sex, and weight of the animals providing the corneas used in a study.

Collection and Transport of Eyes to the Laboratory

15. Eyes are collected by slaughterhouse employees following exsanguination and decapitation of the cattle. To minimize mechanical and other types of damage to the eyes, the eyes should be enucleated as soon as possible after death. To prevent exposure of the eyes to potentially irritating substances, the slaughterhouse employees should not use detergent when rinsing the head of the animal.

16. Eyes should be immersed completely in Hanks' Balanced Salt Solution (HBSS) in a suitably sized container, and transported to the laboratory in such a manner as to minimize deterioration and/or bacterial contamination. Because the eyes are collected during the slaughter process, they might be exposed to blood and other biological substances, including bacteria and other microorganisms. Therefore, it is important to ensure that the risk of contamination is minimized (*e.g.*, by keeping the container containing the eyes on wet ice, by adding antibiotics to the HBSS used to store the eyes during transport [*e.g.*, penicillin at 100 IU/mL and streptomycin

at 100 g/mL]).

17. The time interval between collection of the eyes and use of corneas in the BCOP should be minimized (typically collected and used on the same day) and should be demonstrated to not compromise the assay results. These results are based on the selection criteria for the eyes, as well as the positive and negative control responses. All eyes used in the assay should be from the same group of eyes collected on a specific day.

Selection Criteria for Eyes Used in the BCOP

18. The eyes, once they arrive at the laboratory, are carefully examined for defects including increased opacity, scratches, and neovascularization. Only corneas from eyes free of such defects are to be used.

19. The quality of each cornea is also evaluated at later steps in the assay. Corneas that have an opacity greater than seven opacity units (NOTE: the opacitometer should be calibrated with opacity standards that are used to establish the opacity units) after an initial one hour equilibration period are to be discarded.

20. Each treatment group (test substance, concurrent negative and positive controls) consists of a minimum of three eyes. Three corneas should be used for the negative control corneas in the BCOP assay. Since all corneas are excised from the whole globe, and mounted in the corneal chambers, there is the potential for artifacts from handling upon the control corneal opacity and permeability values. Furthermore, the opacity and permeability values from the negative control corneas are used to correct the test article and positive control-treated corneal opacity and permeability values in the IVIS calculations.

PROCEDURE

Preparation of the Eyes

21. Corneas free of defects are dissected with a 2 to 3 mm rim of sclera remaining to assist in subsequent handling, with care taken to avoid damage to the corneal epithelium and endothelium. Isolated corneas are mounted in specially designed corneal holders that consist of anterior and posterior compartments, which interface with the epithelial and endothelial sides of the cornea, respectively. Both chambers are filled to excess with pre-warmed Eagle's Minimum Essential Medium EMEM (posterior chamber first), ensuring that no bubbles are formed. The device is then equilibrated at 32 ± 1°C for at least one hour to allow the corneas to equilibrate with the medium and to achieve normal metabolic activity, to the extent possible (the approximate temperature of the corneal surface *in vivo* is 32°C).

22. Following the equilibration period, fresh pre-warmed EMEM is added to both chambers and baseline opacity readings are taken for each cornea. Any corneas that show macroscopic tissue damage (*e.g.*, scratches, pigmentation, neovascularization) or an opacity >7 opacity units are discarded. The mean opacity of all equilibrated corneas is calculated. A minimum of three corneas with opacity values close to the median value for all corneas are selected as negative (or solvent) control corneas. The remaining corneas are then distributed into treatment and positive control groups.

23. Because the heat capacity of water is higher than that of air, water provides more stable temperature conditions for incubation. Therefore, the use a water bath for maintaining the corneal

holder and its contents at 32 ± 1ºC is recommended. However, air incubators might also be used, assuming precaution to maintain temperature stability (*e.g.,* by prewarming of holders and media).

Application of the Test Substance

24. Two different treatment protocols are used, one for liquids and surfactants (solids or liquids), and one for non-surfactant solids.

25. Liquids are tested undiluted, while surfactants are tested at a concentration of 10% in a 0.9% sodium chloride solution, distilled water, or other solvent that has been demonstrated to have no adverse effects on the test system. Appropriate justification should be provided for alternative dilution concentrations. Corneas are exposed to liquids and surfactants for 10 minutes. Use of other exposure times should be accompanied by adequate scientific rationale.

26. Non-surfactant solids are typically tested as solutions or suspensions at 20% concentration in a 0.9% sodium chloride solution, distilled water, or other solvent that has been demonstrated to have no adverse effects on the test system. However, solids may also be tested neat by direct application onto the corneal surface using the open chamber method (see paragraph 29). Corneas are exposed to solids for four hours, but as with liquids and surfactants, alternative exposure times may be used with appropriate scientific rationale.

27. Different treatment methods can be used, depending on the physical nature and chemical characteristics (*e.g.,* solids, liquids, viscous vs. non-viscous liquids) of the test substance. The critical factor is ensuring that the test substance adequately covers the epithelial surface and that it is adequately removed during the rinsing steps. A closed-chamber method is typically used for non-viscous to slightly viscous liquid test substances, while an open-chamber method is typically used for semi-viscous and viscous liquid test substances and for neat solids.

28. In the closed-chamber method, sufficient test substance (750 µL) to cover the epithelial side of the cornea is introduced into the anterior chamber through the dosing holes on the top surface of the chamber, and the holes are subsequently sealed with the chamber plugs during the exposure. To ensure that each cornea is exposed for the appropriate time interval, the test substance is applied to the anterior chamber while it is on a diagonal, thereby preventing direct contact with the corneal surface. Once all chambers have been dosed, they are rotated horizontally simultaneously so that the exposure period begins simultaneously.

29. In the open-chamber method, the window-locking ring and glass window from the anterior chamber are removed prior to treatment. The control or test substance (750 µL, or enough test substance to completely cover the cornea) is applied directly to the epithelial surface of the cornea using a micropipet. If a test substance is difficult to pipet, the test substance can be pressure-loaded into a positive displacement pipet to aid in dosing. The pipet tip of the positive displacement pipet is inserted into the dispensing tip of the syringe so that the material can be loaded into the displacement tip under pressure. Simultaneously, the syringe plunger is depressed as the pipet piston is drawn upwards. If air bubbles appear in the pipet tip, the test article is removed (expelled) and the process repeated until the tip is filled without air bubbles. After dosing, the chamber is put back together into the closed system.

Post-Exposure Incubation

30. After the exposure period, the test substance is removed from the anterior chamber and

the epithelium washed at least three times (or until no visual evidence of test substance can be observed) with MEM (containing phenol red). Phenol red-containing medium is used for rinsing since a color change in the phenol red may be monitored to determine the effectiveness of rinsing acidic or alkaline materials. The corneas are washed more than three times if the phenol red is still discolored (yellow or purple), or the test substance is still visible. Once the medium is free of test substance, the corneas are given a final rinse with MEM (without phenol red). The MEM (without phenol red) is used as a final rinse to ensure removal of the phenol red from the anterior chamber prior to the opacity measurement. The anterior chamber is then refilled with fresh MEM without phenol red.

31. For liquids or surfactants, after rinsing, the corneas are incubated for an additional two hours at 32 ± 1ºC. Longer post-exposure time may be useful in certain circumstances and could be considered on a case-by-case basis. Corneas treated with solids are rinsed thoroughly at the end of the four-hour exposure period, but do not require further incubation.

32. At the end of the post-exposure incubation period for liquids and surfactants and at the end of the four-hour exposure period for solids, the opacity of each cornea is recorded. Also, each cornea is observed visually and pertinent observations recorded. Special attention is taken to observe dissimilar opacity patterns, tissue peeling, or residual test substance.

Control Substances

33. Concurrent negative or solvent/vehicle controls and positive controls are included in each experiment.

34. When testing a liquid substance at 100%, a concurrent negative control (*e.g.*, 0.9% sodium chloride solution or distilled water) is included in the BCOP test method so that nonspecific changes in the test system can be detected and to provide a baseline for the assay endpoints. It also ensures that the assay conditions do not inappropriately result in an irritant response.

35. When testing a diluted liquid, surfactant, or solid, a concurrent solvent/vehicle control group is included in the BCOP test method so that nonspecific changes in the test system can be detected and to provide a baseline for the assay endpoints. Only a solvent/vehicle that has been demonstrated to have no adverse effects on the test system can be used.

36. A known ocular irritant is included as a concurrent positive control in each experiment to verify that an appropriate response is induced. As the BCOP assay is being used in this Test Guideline to identify corrosive or severe irritants, ideally the positive control should be a reference substance that induces a severe response in this test method. However, to ensure that variability in the positive control response across time can be assessed, the magnitude of irritant response should not be excessive.

37. Examples of positive controls for liquid test substances are 1% sodium hydroxide, dimethylformamide and 100% ethanol (Although alcohols are a limitation of the BCOP test method, 100% ethanol has consistently produced values that fall within an accepted range for opacity, OD490, and In Vitro Irritancy Score. Although it does not always produce a corrosive or severe irritant response, ethanol is commonly used as a positive control in BCOP because it has been shown to produce consistent results with a response near the borderline of the corrosive/severe irritant response.). An example of a positive control for solid test substances is 20% (weight to volume) imidazole in 0.9% sodium chloride solution.

38. Benchmark substances are useful for evaluating the ocular irritancy potential of unknown chemicals of a specific chemical or product class, or for evaluating the relative irritancy potential of an ocular irritant within a specific range of irritant responses.

Endpoints Measured

39. Opacity is determined by the amount of light transmission through the cornea. Corneal opacity is measured quantitatively with the aid of an opacitometer, resulting in opacity values measured on a continuous scale.

40. Permeability is determined by the amount of sodium fluorescein dye that penetrates all corneal cell layers (*i.e.,* the epithelium on the outer cornea surface through the endothelium on the inner cornea surface). Sodium fluorescein solution (4 or 5 mg/mL when testing liquids or non-surfactant solids, respectively) is added to the anterior chamber of the corneal holder, which interfaces with the epithelial side of the cornea. The concentration of sodium fluorescein that crosses into the posterior corneal chamber, which interfaces with the endothelial side of the cornea, is quantitatively measured with the aid of UV/VIS spectrophotometry. Spectrophotometric measurements evaluated at 490 nm are recorded as optical density (OD_{490}) or absorbance values, which are measured on a continuous scale. The fluorescein permeability values are determined using OD_{490} values based upon a visible light spectrophotometer using a standard 1 cm path length.

41. Alternatively, a 96-well microtiter plate reader may be used provided that; (i) the linear range of the plate reader for determining fluorescein OD_{490} values can be established; and (ii), the correct volume of fluorescein samples are used in the 96-well plate to result in OD_{490} values equivalent to the standard 1 cm path length.

DATA AND REPORTING

Data Evaluation

42. Once the opacity and mean permeability (OD_{490}) values have been corrected for background opacity and the negative control permeability OD_{490} values, the mean opacity and permeability OD_{490} values for each treatment group should be combined in an empirically-derived formula to calculate an *in vitro* score (IVIS) for each treatment group as follows:

IVIS = mean opacity value + (15 x mean permeability OD_{490} value)

43. The opacity and permeability values should also be evaluated independently to determine whether a test substance induced corrosivity or severe irritation through only one of the two endpoints (see Decision Criteria).

Decision Criteria

44. A substance that induces an IVIS $\geq$ 55.1 is defined as a corrosive or severe irritant. As stated in paragraph 1, if the test substance is not identified as an ocular corrosive or severe irritant, additional testing should be conducted for classification and labeling purposes. The BCOP test method has an overall accuracy of 79% (113/143) to 81% (119/147), a false positive rate of 19% (20/103) to 21% (22/103), and a false negative rate of 16% (7/43) to 25% (10/40), when compared to *in vivo* rabbit eye test method data classified according to the EPA (1), EU (2), or GHS (3) classification systems. When substances classified as alcohols, ketones, or solids are excluded from the database, the accuracy of BCOP across the EU, EPA, and GHS classification

systems ranges from 87% (72/83) to 92% (78/85), the false positive rates range from 12% (7/58) to 16% (9/56), and the false negative rates range from 0% (0/27) to 12% (3/26).

45. Even if an ocular corrosive or severe irritant classification is not obtained for a test substance, BCOP data can be useful, in conjunction with test data from the *in vivo* rabbit eye test or from a valid *in vitro* test, to further evaluate the usefulness and limitations of the BCOP test method for identifying non-severe irritants and non-irritants (see Guidance Document on the use of *in vitro* ocular toxicity test methods).

46. Benchmark substances are recommended for aiding in the evaluation of responses of test substances of different product or chemical classes.

Study Acceptance Criteria

47. A test is acceptable if the positive control gives an IVIS that falls within two standard deviations of the current historical mean, which is to be updated at least every three months, or each time an acceptable test is conducted in laboratories where tests are conducted infrequently (*i.e.*, less than once a month). The negative or solvent/vehicle control responses should result in opacity and permeability values that are less than the established upper limits for background opacity and permeability values for bovine corneas treated with the respective negative or solvent/vehicle control.

Test Report

48. The test report should include the following information, if relevant to the conduct of the study:

Test and Control Substances;

- Chemical name(s) such as the structural name used by the Chemical Abstracts Service (CAS), followed by other names, if known;
- The CAS Registry Number (RN), if known;
- Purity and composition of the substance or preparation (in percentage(s) by weight), to the extent this information is available;
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study;
- Treatment of the test/control substances prior to testing, if applicable (*e.g.*, warming, grinding);
- Stability, if known.

Information Concerning the Sponsor and the Test Facility;

- Name and address of the sponsor, test facility and study director;
- Identification of the source of the eyes (*i.e.*, the facility from which they were collected);
- Storage and transport conditions of eyes (*e.g.*, date and time of eye collection, time interval prior to initiating testing, transport media and temperature conditions, any antibiotics used);
- If available, specific characteristics of the animals from which the eyes were collected (*e.g.*, age, sex, weight of the donor animal).

Justification of the Test Method and Protocol Used

Test Method Integrity;

The procedure used to ensure the integrity (*i.e.*, accuracy and reliability) of the test method over time (*e.g.*, periodic testing of proficiency substances, use of historical negative and positive control data).

Criteria for an Acceptable Test;

Acceptable concurrent positive and negative control ranges based on historical data;

If applicable, acceptable concurrent benchmark control ranges based on historical data.

Test Conditions;

Description of test system used;

Type of corneal holder used;

Calibration information for devices used for measuring opacity and permeability (*e.g.*, opacitometer and spectrophotometer);

Information on the bovine corneas used, including statements regarding their quality;

Details of test procedure used;

Test substance concentration(s) used;

Description of any modifications of the test procedure;

Reference to historical data of the model (*e.g.*, negative and positive controls, proficiency substances, benchmark substances);

Description of evaluation criteria used.

Results;

Tabulation of data from individual test samples (*e.g.*, opacity and OD_{490} values and calculated IVIS for the test substance and the positive, negative, and benchmark controls [if included], reported in tabular form, including data from replicate repeat experiments as appropriate, and means ± the standard deviation for each experiment);

Description of other effects observed.

Discussion of the Results

Conclusion

A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant Studies;

This statement indicates all inspections made during the study, and the dates any results were reported to the Study Director. This statement also serves to confirm that the final report reflects the raw data.

If GLP-compliant studies are performed the additional reporting requirements should be followed (17).

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ANNEX I

DEFINITIONS

Accuracy: The closeness of agreement between test method results and accepted reference values. It is a measure of test method performance and one aspect of "relevance." The term is often used interchangeably with "concordance", to mean the proportion of correct outcomes of a test method.

Benchmark substance: A substance used as a standard for comparison to a test substance. A benchmark substance should have the following properties; (i), a consistent and reliable source(s); (ii), structural and functional similarity to the class of substances being tested; (iii), known physical/chemical characteristics; (iv), supporting data on known effects; and (v), known potency in the range of the desired response

Cornea: The transparent part of the front of the eyeball that covers the iris and pupil and admits light to the interior.

Corneal opacity: Measurement of the extent of opaqueness of the cornea following exposure to a test substance. Increased corneal opacity is indicative of damage to the cornea. Opacity can be evaluated subjectively as done in the Draize rabbit eye test, or objectively with an instrument such as an "opacitometer."

Corneal permeability: Quantitative measurement of damage to the corneal epithelium by a determination of the amount of sodium fluorescein dye that passes through all corneal cell layers.

EPA Category 1: Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days.

EU Category R41: Production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.

False negative rate: The proportion of all positive substances falsely identified by a test method as negative. It is one indicator of test method performance.

False positive rate: The proportion of all negative substances that are falsely identified by a test method as positive. It is one indicator of test method performance.

Globally Harmonized System (GHS): A classification system presented by the United Nations that provides (a) a harmonized criteria for classifying substances and mixtures according to their health, environmental and physical hazards, and (b) harmonized hazard communication elements, including requirements for labeling and safety data sheets.

GHS Category 1: Production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.

Good Laboratory Practices (GLP): Regulations promulgated by a number of countries and national regulatory bodies that describe record keeping and quality assurance procedures for laboratory records that will be the basis for data submissions to regulatory authorities. Also the

subject of the OECD Series on "Principles of Good Laboratory Practice and Compliance Monitoring".

Hazard: Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub) population is exposed to that agent.

***In Vitro* Irritancy Score:** An empirically-derived formula used in the BCOP assay whereby the mean opacity and mean permeability values for each treatment group are combined into a single *in vitro* score for each treatment group. The *IVIS* = mean opacity value + (15 x mean permeability value).

Negative control: An untreated replicate containing all components of a test system. This sample is processed with test substance-treated samples and other control samples to determine whether the solvent interacts with the test system.

Non-irritant: Substances that are not classified as EPA Category I, II, or III; EU Category R41 or R36; or GHS Category 1, 2A, or 2B ocular irritants.

Ocular corrosive: (a) A substance that causes irreversible tissue damage to the eye. (b) Substances that are classified as GHS Category 1, EPA Category I, or EU Category R41 ocular irritants.

Ocular irritant: (a) A substance that produces a reversible change in the eye following application to the anterior surface of the eye. (b) Substances that are classified as EPA Category II or III, EU Category R36, or GHS Category 2A or 2B ocular irritants.

Ocular severe irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye that does not resolve within 21 days of application or causes serious physical decay of vision. (b) Substances that are classified as GHS Category 1, EPA Category I, or EU Category R41 ocular irritants.

Opacitometer: An instrument used to measure "corneal opacity" by quantitatively evaluating light transmission through the cornea. The typical instrument has two compartments, each with its own light source and photocell. One compartment is used for the treated cornea, while the other is used to calibrate and zero the instrument. Light from a halogen lamp is sent through a control compartment (empty chamber without windows or liquid) to a photocell and compared to the light sent through the experimental compartment, which houses the chamber containing the cornea, to a photocell. The difference in light transmission from the photocells is compared and a numeric opacity value is resented on a digital display.

Positive control: A replicate containing all components of a test system and treated with a substance known to induce a positive response. To ensure that variability in the positive control response across time can be assessed, the magnitude of the severe response should not be excessive.

Reliability: Measures of the extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol. It is assessed by calculating intra- and inter-laboratory reproducibility and intra-laboratory repeatability.

Solvent/vehicle control: An untreated sample containing all components of a test system, including the solvent or vehicle that is processed with the test substance-treated and other control

samples to establish the baseline response for the samples treated with the test substance dissolved in the same solvent or vehicle. When tested with a concurrent negative control, this sample also demonstrates whether the solvent or vehicle interacts with the test system.

Tiered testing: A stepwise testing strategy where all existing information on a test substance is reviewed, in a specified order, using a weight of evidence process at each tier to determine if sufficient information is available for a hazard classification decision, prior to progression to the next tier. If the irritancy potential of a test substance can be assigned based on the existing information, no additional testing is required. If the irritancy potential of a test substance cannot be assigned based on the existing information, a step-wise sequential animal testing procedure is performed until an unequivocal classification can be made.

Validated test method: A test method for which validation studies have been completed to determine the relevance (including accuracy) and reliability for a specific purpose. It is important to note that a validated test method may not have sufficient performance in terms of accuracy and reliability to be found acceptable for the proposed purpose.

Weight-of-evidence: The process of considering the strengths and weaknesses of various pieces of information in reaching and supporting a conclusion concerning the hazard potential of a substance.

ANNEX II

PROFICIENCY STANDARDS FOR THE BCOP TEST METHOD (TO BE PROVIDED)

ANNEX III

THE BCOP CORNEAL HOLDER

The BCOP corneal holders are made of an inert material (*e.g.*, polypropylene). The holders are comprised of two halves (an anterior and posterior chamber), and have two similar cylindrical internal chambers. Each chamber holds a volume of 5 mL and terminates in a glass window, through which opacity measurements are recorded. Each of the inner chambers is 1.7 cm in diameter and 2.2 cm in depth. An o-ring located on the posterior chamber is used to prevent leaks. The corneas are placed endothelial side down on the o-ring of the posterior chamber and the anterior chamber placed on the epithelial side of the cornea. The chambers are maintained in place by three stainless screws located on the outer edges of the chamber. The end of each chamber houses a glass window which can be removed for easy access to the cornea. An o-ring is also located between the glass window and the chamber to prevent leaks. Two holes on the top of each chamber permit introduction and removal of medium and test compounds. They are closed with rubber caps during the treatment and incubation periods.